MSD® MULTI-SPOT Assay System

Kidney Injury Panel 5 (human) Kit

1-Plate Kit	K15188D-1
5-Plate Kit	K15188D-2
25-Plate Kit	K15188D-4

www.mesoscale.com®

MSD Toxicology Assays

Kidney Injury Panel 5 (human) Kit

Albumin, B2M, Cystatin C, EGF, NGAL/LCN2, OPN, UMOD

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Introduction

Albumin is the most abundant serum protein (50–60% of total serum protein). It acts as a transport protein for hemin and fatty acids.[1] Albumin is produced in the liver and secreted into the bloodstream. Damage to the kidney can lead to albuminuria, secretion of albumin into the urine.[2-4] Increased albumin measurements in serum may also be due to anabolic steroids, androgens, growth hormone, and insulin.[1,2]

Beta-2-Microglobulin (B2M) is a 12 kDa, secreted, and ubiquitously expressed protein. B2M is normally filtered in the kidneys through the glomerulus, then reabsorbed and metabolized in the proximal tubules.[5] Under normal conditions, trace amounts of B2M are excreted in the urine. In the event of kidney toxicity, an elevated level of B2M is associated with glomerular injury and renal tube damage.[6,7]

Cystatin C is a 13 kDa, non-glycosylated proteinase inhibitor and a member of the type 2 cystatin superfamily. Studies have shown that cystatin C is a more precise indicator of kidney damage than traditional clinical markers such as BUN (blood urea nitrogen) or serum creatinine.[8,9] Evidence supports a strong correlation between elevated blood levels of cystatin C and kidney dysfunction.[8,9] Studies also indicate that cystatin C is a more sensitive measure of glomerular filtration rate (GFR) than creatinine clearance.[9]

Epithelial growth factor (EGF) plays an important role in cell regulation and proliferation. EGF is down-regulated in children with renal parenchyma and is a biomarker of tubulointerstitial damage in human obstructive nephropathy.[10] The level of EGF increases in ischemic acute renal failure.[11]

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2 (LCN2), is a 25 kDa glycoprotein secreted from specific granules of activated human neutrophils. NGAL is expressed in most tissues and is induced in epithelial cells upon inflammation.[12] Elevated urinary and serum NGAL levels have been observed in patients with established renal failure.[13]

Osteopontin (OPN), also known as Eta-1, is a pleiotropic cytokine, most often expressed in bone, kidney, and epithelial tissues. OPN is overexpressed in a variety of cancers and in patients with nephritis and acute kidney injury (AKI).[14-16] OPN has been reported to be a prognosis biomarker in patients who need renal replacement therapy after acute kidney injury.[17]

Uromodulin (UMOD), also known as Tamm-Horsfall glycoprotein (THP), is a 68 kDa glycoprotein, produced in the thick ascending limb of the loop of Henle. It is the most abundant protein in normal, healthy urine. Increased expression of UMOD can cause it to leak into the renal interstitium resulting in the recruitment and stimulation of immune response, causing inflammation and kidney damage.[18-20] UMOD deposits have been observed in renal biopsies of tubulointerstitial disease.[21]

Principle of the Assay

MSD toxicology assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. The assays in the Kidney Injury Panel 5 (human) Kit are sandwich immunoassays (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent MSD SULFO-TAG™ labels over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

1. Albumin
2. B2M
3. Cystatin C
4. EGF
5. NGAL
6. OPN
7. UMOD

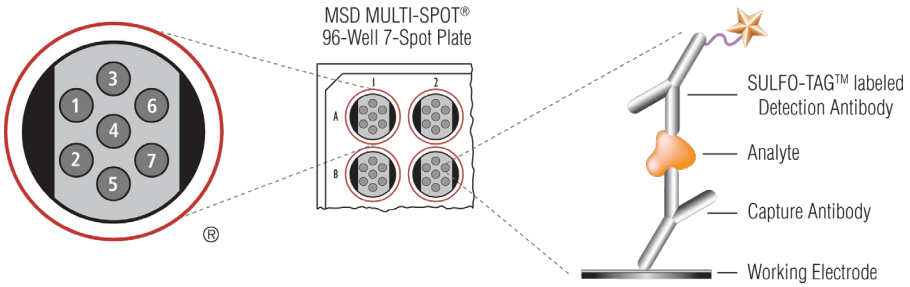

Figure 1. Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

CAUTION: Some of the biomarkers in the Kidney Injury Panel 5 (human) Kit, especially albumin, are found at high concentrations on skin and in saliva and other bodily fluids. It is very important to use extreme care (i.e., use gloves, lab coats, and face shields; use separate plate seals for each incubation and wash; and change pipette tips after each use) when running this assay to avoid contamination that might cause high background, poor precision, or unexpected results.

Reagents Supplied

Product Description	Storage	Catalog #	Size	Quantity per Kit		
				K15188D-1	K15188D-2	K15188D-4
MULTI-SPOT® 96-Well Kidney Injury Panel 5 (human) Plate	2–8°C	N75188A-1	7-spot	1 plate	5 plates	25 plates
SULFO-TAG Anti-hu Albumin Antibody (50X)[1]	2–8°C	D21IK-2	75 µL	1 vial		
		D21IK-3	375 µL		1 vial	5 vials
SULFO-TAG Anti-hu B2M Antibody (50X)[1]	2–8°C	D21KA-2	75 µL	1 vial		
		D21KA-3	375 µL		1 vial	5 vials
SULFO-TAG Anti-hu Cystatin C Antibody (50X)[1]	2–8°C	D21LM-2	75 µL	1 vial		
		D21LM-3	375 µL		1 vial	5 vials
SULFO-TAG Anti-hu EGF Antibody (50X)[1]	2–8°C	D21KM-2	75 µL	1 vial		
		D21KM-3	375 µL		1 vial	5 vials
SULFO-TAG Anti-hu NGAL Antibody (50X)[1]	2–8°C	D21KN-2	75 µL	1 vial		
		D21KN-3	375 µL		1 vial	5 vials
SULFO-TAG Anti-hu OPN Antibody (50X)[1]	2–8°C	D21KO-2	75 µL	1 vial		
		D21KO-3	375 µL		1 vial	5 vials
SULFO-TAG Anti-hu UMOD Antibody (50X)[1]	2–8°C	D21KP-2	75 µL	1 vial		
		D21KP-3	375 µL		1 vial	5 vials
Kidney Injury Panel 5 (human) Calibrator Blend (20X)[2]	≤-70°C	C0188-2	20 µL	1 vial	5 vials	25 vials
Diluent 37	≤-10°C	R50AF-3	25 mL	2 bottles		
		R50AF-6	125 mL		2 bottles	10 bottles
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer)	RT	R93AA-2	250 mL	1 kit	1 kit	5 kits
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing or MSD Wash Buffer (Catalog No. R61AA-1)
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker (rotary) capable of shaking at 500–1,000 rpm
- ❑ Deionized water
- ❑ Vortex mixer

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.
[2] **Biohazard Statement:** This product was derived from human material and should be considered potentially infectious. Appropriate precautions should be used when handling this material.

Safety

Use safe laboratory practices: wear gloves, safety glasses, and lab coats when handling assay components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service or at www.mesoscale.com.

Best Practices

- Bring frozen diluent to room temperature in a 22-25°C water bath. Thaw frozen calibrator (when applicable) on wet ice.
- Prepare Calibrator Standards and samples in polypropylene microcentrifuge tubes. Use a fresh pipette tip for each dilution and mix by vortexing after each dilution.
- Avoid prolonged exposure of the detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light (except for direct sunlight).
- Avoid bubbles in wells during all pipetting steps as they may lead to variable results. Bubbles introduced when adding Read Buffer may interfere with signal detection.
- Use reverse pipetting when necessary to avoid the introduction of bubbles. For empty wells, pipette gently to the bottom corner.
- Plate shaking should be vigorous, with a rotary motion between 500 and 1,000 rpm. Binding reactions may reach equilibrium sooner if you use shaking at the middle of this range (~700 rpm) or above.
- When using an automated plate washer, rotate the plate 180 degrees between wash steps to improve assay precision.
- Gently tap the plate on a paper towel to remove residual fluid after washing.
- If an incubation step needs to be extended, leave the sample or detection antibody solution in the plate to keep the plate from drying out.
- Remove the plate seal prior to reading the plate.
- Make sure that the read buffer is at room temperature when adding to a plate.
- Do not shake the plate after adding the read buffer.
- To improve inter-plate precision, keep time intervals consistent between adding the read buffer and reading the plate. Unless otherwise directed, read the plate as soon as possible after adding the read buffer.
- If the sample results are above the top of the calibration curve, dilute the samples and repeat the assay.
- When running a partial plate, seal the unused sectors to avoid contaminating unused wells. Remove all seals before reading. The uncoated wells of a partially used plate may be sealed and stored up to 30 days at 2–8°C in the original foil pouch with desiccant. You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

Important: Upon first thaw, separate Diluent 37 into aliquots appropriate to the size of your needs before refreezing.

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Prepare Blocker A Solution

Follow the Blocker A instructions included in the kit.

Prepare Calibrator Solutions

MSD supplies blended calibrator for the Kidney Injury Panel 5 (human) Kit at 20-fold higher concentration than the recommended highest calibrator. We recommend a 7-point standard curve with 4-fold serial dilution steps and a zero calibrator blank. Signals from the blank should be excluded when generating the curve. Thaw the stock calibrator on ice, then add to diluent at room temperature to make the standard curve solutions. For the actual concentration of each calibrator in the blend, refer to the certificate of analysis (COA) supplied with the kit or available at www.mesoscale.com.

To prepare 7 standard solutions plus a zero calibrator blank for up to 3 replicates:

1) Prepare the highest standard by adding 15 µL of stock calibrator to 285 µL of Diluent 37. Mix well.
2) Prepare the next standard by transferring 60 µL of the highest standard to 180 µL of Diluent 37. Mix well. Repeat 4-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 37 as the blank.

Dilute Samples

For human urine samples, MSD recommends a 500-fold dilution in Diluent 37; however, you may adjust dilution factors for the sample set under investigation. For some samples, Cystatin C might require lower dilution (50 to 100-fold) and Albumin might require higher dilution (more than 500-fold).

We recommend diluting human serum samples 500-fold for all analytes except albumin (320,000-fold).

To dilute sample 500-fold:

1) Add 10 µL of sample to 490 µL of Diluent 37 (50-fold dilution)
2) Add 20 µL of the diluted sample to 180 µL of Diluent 37 (10-fold dilution)

Prepare Detection Antibody Solution

MSD provides each detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For one plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-hu Albumin Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu B2M Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu Cystatin C Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu EGF Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu NGAL Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu OPN Antibody
- ❑ 60 µL of 50X SULFO-TAG Anti-hu UMOD Antibody
- ❑ 2,580 µL of Diluent 37

You may omit detection antibody for any analyte not being measured; add 60 µL of Diluent 37 for each omitted antibody.

Prepare Wash Buffer

MSD Wash Buffer (Catalog No. R61AA-1) comes as a 20X stock solution. Dilute the stock solution to 1X before use. PBS + 0.05% Tween-20 (PBS-T) can be used instead.

For one plate, combine:

- ❑ 15 mL of MSD Wash Buffer (20X)
- ❑ 285 mL of deionized water

1X MSD Wash Buffer can be stored at room temperature for up to two weeks.

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For one plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container for up to one month.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Note: Follow **Reagent Preparation** before beginning this assay protocol.

STEP 1: Add Blocker A Solution

- ❑ Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 30 minutes with vigorous shaking (500–1,000 rpm) at room temperature.

STEP 2: Wash and Add Sample

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

  CAUTION: Use extreme care when running this assay to avoid contamination.

  You may prepare detection antibody solution during incubation.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL of 1X detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (500–1,000 rpm) at room temperature.

  You may prepare diluted read buffer during incubation.

STEP 4: Wash and Read

- ❑ Wash the plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on an MSD instrument. Incubation in read buffer is not required before reading the plate.

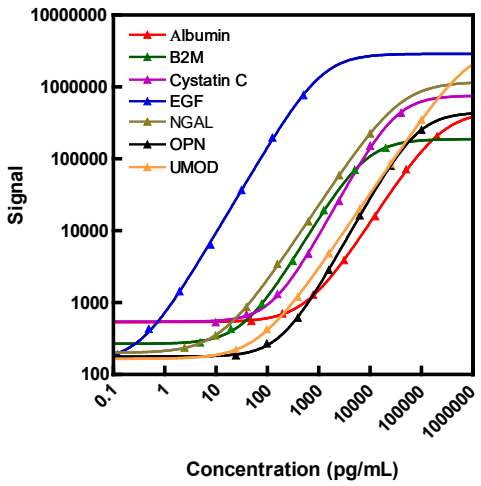
Analysis of Results

Run at least one set of calibrators in duplicate to generate the standard curve. The standard curve is modeled using least squares fitting algorithms so that signals from the calibrators can be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) which allows accurate quantification without the need for dilution in many cases. The MSD DISCOVERY WORKBENCH® analysis software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of two replicates of standards. For each kit lot, refer to the COA for the actual concentration of the calibrator.

Albumin		
Conc. (pg/mL)	Average Signal	%CV
0	508	2.6
49	553	4.6
195	706	3.0
781	1,291	13.6
3,125	3,905	4.2
12,500	15,889	4.3
50,000	71,585	1.2
200,000	205,950	6.0



B2M		
Conc. (pg/mL)	Average Signal	%CV
0	225	1.2
4.9	279	5.5
20	432	5.4
78	968	2.7
313	3,800	2.7
1,250	19,204	2.7
5,000	70,301	4.4
20,000	141,644	3.5

Cystatin C		
Conc. (pg/mL)	Average Signal	%CV
0	474	3.0
9.8	531	8.2
39	689	7.6
156	1,315	7.3
625	4,787	9.1
2,500	25,871	12.7
10,000	152,410	8.3
40,000	437,126	3.9

EGF		
Conc. (pg/mL)	Average Signal	%CV
0	95	3.1
0.12	190	6.1
0.49	432	4.9
2.0	1,439	2.3
7.8	6,402	6.0
31	36,848	3.0
125	197,010	4.8
500	766,992	2.5

NGAL		
Conc. (pg/mL)	Average Signal	%CV
0	144	4.6
2.4	237	13.1
9.8	350	3.7
39	867	2.5
156	3,446	2.9
625	13,503	7.2
2,500	58,872	5.0
10,000	224,648	0.5

OPN		
Conc. (pg/mL)	Average Signal	%CV
0	150	5.6
24	184	2.0
98	271	5.2
391	616	3.7
1,563	2,850	9.0
6,250	16,314	1.6
25,000	79,451	5.1
100,000	253,388	2.5

UMOD		
Conc. (pg/mL)	Average Signal	%CV
0	150	4.3
24	219	6.2
98	426	2.8
391	1,197	4.8
1,563	4,829	4.9
6,250	20,285	6.4
25,000	85,773	6.4
100,000	346,943	7.6

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank). The LLOD shown below was calculated based on 36 runs.

	Albumin	B2M	Cystatin C	EGF	NGAL	OPN	UMOD
Average LLOD (pg/mL)	141	6.1	27	0.13	2.9	90	26
LLOD Range (pg/mL)	28–359	1.3–12	5.4–81	0.036–0.26	1.1–10	37–355	12–107

Precision

Human urine-based control samples with high, medium, and low levels of each analyte were measured using a minimum of two replicates on 11 runs over five days.

Average intra-run %CV is the average %CV of the control replicates on an individual run.

Inter-run %CV is the variability of controls across 11 runs.

	Control	Runs	Average Conc. (pg/mL)	Average Intra-run %CV	Inter-run %CV
Albumin	High	11	82,714	7.0	10.4
	Mid	11	15,052	6.8	13.9
	Low	11	2.688	3.4	9.5
B2M	High	11	3.728	6.5	13.8
	Mid	11	563	3.4	8.6
	Low	11	119	4.7	10.1
Cystatin C	High	11	32.816	6.2	23.6
	Mid	11	4,364	4.3	13.9
	Low	11	823	4.1	14.7
EGF	High	11	134	8.2	14.1
	Mid	11	40	7.3	14.5
	Low	11	2.4	6.1	12.2
NGAL	High	11	3,105	5.2	9.7
	Mid	11	2,328	3.4	10.9
	Low	11	41	6.2	16.6
OPN	High	11	9,380	6.5	18.4
	Mid	11	3,157	7.6	16.0
	Low	11	349	7.1	19.9
UMOD	High	11	28,985	4.9	14.3
	Mid	11	5,660	4.1	13.4
	Low	11	1,254	3.1	10.5

Dilution Linearity

To assess linearity, normal human urine samples were diluted 125-fold, 250-fold, 500-fold, 1000-fold, and 2000-fold before testing. Percent recovery at each dilution was calculated by dividing the measured concentration by the expected concentration (concentration at 500-fold dilution). The average percent recovery shown below was calculated from samples with values above the LLOD. % Recovery=measured/expected∗100

		Albumin		B2M		Cystatin C		EGF	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Urine (N=10)	125	120	87–161	110	86–135	97	85–119	109	99–116
	250	119	100–134	100	88–120	100	87–114	106	98–112
	1,000	79	59–97	96	95–105	89	84–92	98	87–126
	2,000	73	54–83	110	98–124	91	83–98	100	87–114

		NGAL		OPN		UMOD	
Sample Type	Fold Dilution	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range	Average % Recovery	% Recovery Range
Urine (N=10)	125	100	94–104	167	143–186	108	100–115
	250	100	94–104	139	113–167	103	99–108
	1,000	97	90–101	64	60–72	95	92–99
	2,000	105	96–113	54	52–56	101	92–111

Spike Recovery

Normal human urine samples were diluted 500-fold then spiked with calibrators at multiple levels throughout the range of the assay. The average percent recovery shown below was calculated from samples with values above the LLOD.

% Recovery=measured/expected∗100

	Albumin			B2M			Cystatin C		
Sample Type	Spike Conc. (pg/mL)	Average % Recovery	% Recovery Range	Spike Conc. (pg/mL)	Average % Recovery	% Recovery Range	Spike Conc. (pg/mL)	Average % Recovery	% Recovery Range
Urine (N=8)	625	95	83–110	63	98	89–108	125	97	87–113
	2,500	96	86–104	250	87	78–96	500	92	81–107
	10,000	96	88–105	1,000	98	85–107	2,000	93	87–103
	40,000	90	75–97	4,000	96	83–103	8,000	94	71–107
	160,000	99	95–111	16,000	83	77–92	32,000	102	92–122

	EGF			NGAL			OPN		
Sample Type	Spike Conc. (pg/mL)	Average % Recovery	% Recovery Range	Spike Conc. (pg/mL)	Average % Recovery	% Recovery Range	Spike Conc. (pg/mL)	Average % Recovery	% Recovery Range
Urine (N=8)	1.6	94	90–102	31	97	88–104	313	98	88–106
	6.3	89	75–104	125	91	79–99	1,250	96	86–106
	25	98	84–108	500	97	83–104	5,000	97	85–108
	100	97	82–109	2,000	90	75–98	20,000	91	75–98
	400	98	91–111	8,000	100	95–108	80,000	95	86–106

	UMOD		
Sample Type	Spike Conc. (pg/mL)	Average % Recovery	% Recovery Range
Urine (N=8)	313	97	85–107
	1,250	90	78–96
	5,000	93	83–102
	20,000	85	73–93
	80,000	93	86–104

Specificity

To assess specificity of the detection antibodies, the Kidney Injury Panel 5 (human) was run using blended calibrators with individual detection antibodies and using blended detction antibodies with individual calibrators (40 ng/mL albumin; 3.0 ng/mL B2M; 18 ng/mL cystatin C; 0.05 ng/mL EGF; 1.0 ng/mL NGAL; 12 ng/mL OPN; 14 ng/mL UMOD). No significant cross-reactivity (>0.5%) was observed, except with binding of the UMOD detection antibody to the OPN calibrator (<3%). The extent of binding of the UMOD detection antibody to the OPN calibrator varies due to inconsistent behavior of diluent raw materials.

Stability

Kit components were tested for freeze-thaw stability. Results (not shown) demonstrated that blended calibrator is stable for one freeze-thaw cycle, and controls can go through three freeze-thaw cycles without affecting assay performance.

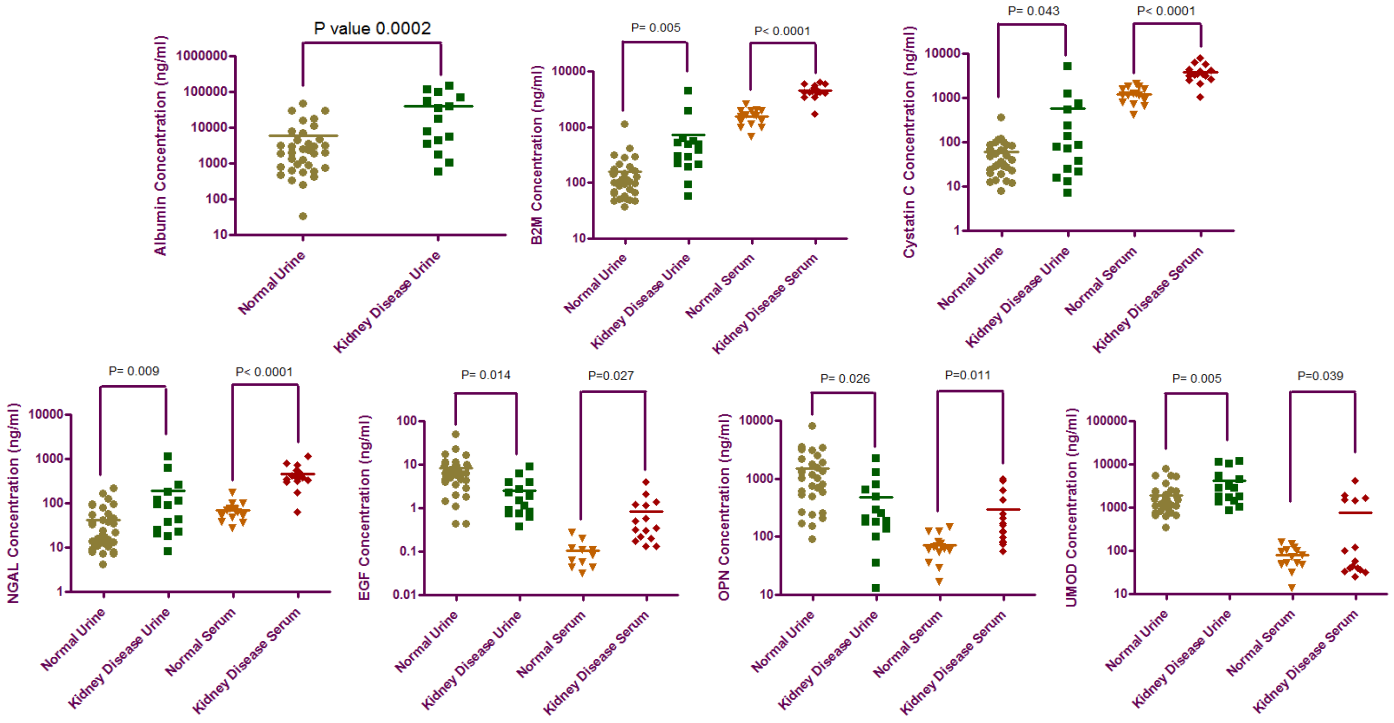
Tested Samples

Normal and disease samples (both urine and serum), were diluted 500-fold and tested with the Kidney Injury Panel 5 (human). Median and range of concentrations for each sample set are displayed below. Concentrations are corrected for sample dilution.

Sample Type	Statistic	Albumin	B2M	Cystatin C	EGF	NGAL	OPN	UMOD
Normal Urine*	Median (ng/mL)	2,002	106	38	6.5	18	1,031	1,350
	Range (ng/mL)	<LLOD–48,757	38–1,130	<LLOD–370	0.43– 51	4.2–225	<LLOD–8,146	347–7,846
	Number of Samples	35	35	35	35	35	35	35
	Samples above LLOD	33	35	30	35	35	30	35
Kidney Disease Urine*	Median (ng/mL)	17,822	398	79	1.5	93	214	2897
	Range (ng/mL)	597–150,866	59–4,557	<LLOD–5,410	0.38–9.3	8.2–1,148	<LLOD–2,265	876–12,062
	Number of Samples	15	15	15	15	15	15	15
	Samples above LLOD	15	15	14	15	15	13	15
Normal Serum*	Median (ng/mL)	**	1,632	1,169	0.085	63.2	67	74
	Range (ng/mL)	**	673–2,637	423–2,071	<LLOD–0.28	28–175	<LLOD–150	14–158
	Number of Samples	–	15	15	15	15	15	15
	Samples above LLOD	–	15	15	9	15	12	15
Kidney Disease Serum*	Median (ng/mL)	**	4,315	3,450	0.35	411	158	58
	Range (ng/mL)	**	1,728–6,400	1,071–7,929	0.13–4.0	63–1,149	56–986	26–4,229
	Number of Samples	–	15	15	15	15	15	15
	Samples above LLOD	–	15	15	15	15	15	15

**Clinical information associated with normal and kidney disease samples was not available.*

***Sample signal exceeds the top of standard curve at 500-fold dilution signal. Albumin testing in human serum requires >500-fold dilution.*



Assay Components

Calibrators

The assay calibrator blend uses the following recombinant human proteins:

Cystatin C (residues 27-146) expressed in a human cell line

OPN (residues 17-300) expressed in a mouse cell line

NGAL (residues 21-198) expressed in a mouse cell line

EGF (residues 971-1023) expressed in *E. coli*

Albumin, B2M, and UMOD are native human proteins.

Antibodies

	Source Species	
Analyte	MSD Capture Antibody	MSD Detection Antibody
Albumin	Mouse Monoclonal	Mouse Monoclonal
B2M	Mouse Monoclonal	Mouse Monoclonal
Cystatin C	Mouse Monoclonal	Goat Polyclonal
EGF	Mouse Monoclonal	Goat Polyclonal
NGAL	Mouse Monoclonal	Goat Polyclonal
OPN	Mouse Monoclonal	Goat Polyclonal
UMOD	Mouse Monoclonal	Sheep Polyclonal

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Summary Protocol

Kidney Injury Panel 5 (human) Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Kidney Injury Panel 5 (human) assays.

Sample and Reagent Preparation

CAUTION: Some of the biomarkers in the Kidney Injury Panel 5 (human) Kit, especially albumin, are found at high concentrations on skin and in saliva and other bodily fluids. It is very important to use extreme care (i.e., use gloves, lab coats, and face shields; use separate plate seals for each incubation and wash; and change pipette tips after each use) when running this assay to avoid contamination that might cause high background, poor precision, or unexpected results.

- ❑ Bring all reagents to room temperature and thaw the calibrator on ice.
- ❑ Prepare Blocker A solution.
- ❑ Prepare 7 standard solutions using the supplied calibrator:
  - Dilute the stock calibrator 20-fold in Diluent 37.
  - Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.
- ❑ Dilute samples 500-fold in Diluent 37 before adding to the plate.
- ❑ Prepare combined detection antibody solution by diluting each stock detection antibody 50-fold in Diluent 37.
- ❑ Prepare 2X Read Buffer T by diluting 4X Read Buffer T 2-fold with deionized water.

STEP 1: Add Blocker A Solution

- ❑ Add 150 µL/well of Blocker A solution.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 30 minutes.

STEP 2: Wash and Add Sample

- ❑ Wash plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 50 µL/well of sample (standards, controls, or unknowns).
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 2 hours.

STEP 3: Wash and Add Detection Antibody Solution

- ❑ Wash plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 25 µL/well of 1X detection antibody solution.
- ❑ Incubate at room temperature with vigorous shaking (500–1,000 rpm) for 2 hours.

STEP 4: Wash and Read Plate

- ❑ Wash plate 3 times with 300 µL/well of 1X MSD Wash Buffer or PBS-T.
- ❑ Add 150 µL/well of 2X Read Buffer T.
- ❑ Analyze plate on an MSD instrument.

Plate Diagrams